L Number	Hits	Search Text	DB	Time stamp
1	19	situ near2 hybridization near2 flourescen$8	USPAT; US-PGPUB; DERWENT	2003/09/11 09:23
2	25	situ same preamplifier	USPAT; US-PGPUB; DERWENT	2003/09/11 09:23
3	4	(situ same preamplifier) and 435/6	USPAT; US-PGPUB; DERWENT	2003/09/11 09:45
4	36	Nolte-f$.in.	USPAT; US-PGPUB; DERWENT	2003/09/11 09:45
5	0	Nolte-f$.in. same dna	USPAT; US-PGPUB; DERWENT	2003/09/11 09:45
6	1	Nolte-f$.in. and dna	USPAT; US-PGPUB; DERWENT	2003/09/11 09:45
7	6191	"in situ hybridization" or ish	USPAT; US-PGPUB; DERWENT	2003/09/11 09:53
8	439	"anti dig"	USPAT; US-PGPUB; DERWENT	2003/09/11 09:54
9	21	("in situ hybridization" or ish) same "anti dig"	USPAT; US-PGPUB; DERWENT	2003/09/11 10:26
10	2	"5858662"	USPAT; US-PGPUB; DERWENT	2003/09/11 10:26

(FILE 'HOME' ENTERED AT 09:30:40 ON 11 SEP 2003)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 09:30:54 ON 11 SEP 2003

```
L1        821568 S IN SITU HYBRIDIZATION OR ISH OR FISH
L2         65449 S AMPLIFIER OR PREAMPLIFIER
L3           107 S L1 AND L2
L4            61 DUP REM L3 (46 DUPLICATES REMOVED)
L5            66 S L1 (P) L2
L6            34 DUP REM L5 (32 DUPLICATES REMOVED)
L7             9 S L6 AND (DNA OR RNA OR NUCLEIC)

=>
```

US-PAT-NO: 5858662

DOCUMENT-IDENTIFIER: US 5858662 A

TITLE: Diagnosis of Williams syndrome and Williams syndrome cognitive profile by analysis of the presence or absence of a LIM-kinase gene

---------- KWIC ---------

Drawing Description Text - DRTX (7):
FIGS. 6A-6H. In situ hybridization analysis of LIMK1 expression in the nervous system of a Carnegie stage 20 (50 day postovulatory) human embryo. A 625-bp LIMK1 cRNA probe was labeled with DIG-UTP and visualized using anti-DIG alkaline phosphatase antibody. (A) Transverse section through rhombencephalon/medulla, fourth ventricle. LIMK1 expression is seen in the ependymal layer of the fourth ventricle and a lower level of expression extends into the mantle layer. The arrow indicates expression in the medial accessory olivary nucleus on either side of the midline; this area is shown in greater detail in C. (B) Similar section to (A) hybridized with the sense-strand cRNA probe as a negative control. (C) Medial accessory olivary nuclei shown in the center of (A). (D) Transverse section through the cerebellum (c) showing a high level of ependymal expression in the corpus cerebelli (fourth ventricle on the right and ectoderm on the left). Some expression is visible in the mesenchyme adjacent to the ectoderm, in particular in the presumptive dentate nucleus (arrow). (E) Transverse section through the cervical spinal cord showing generalized expression in the dorsal (top) part of

the spinal cord and
single-cell staining more ventrally (right). There is also expression in the
dorsal root ganglia (d). (F) Section through the wall of the mesencephalon
(the ventricle is on the far right); the ependymal layer is on the right and
heavily stained, and the mantle layer in the center-left shows many cells
expressing LIMK1. An arrow indicates the sulcus limitans. (G) Higher
magnification of (E), showing the mid-area of the spinal cord, demonstrates a
low level of confluent expression in the ependymal layer (right), widespread
single-cell staining in the mantle layer (center), and lack of expression in
the marginal layer (left). (H) Transverse section through the fifth nerve
ganglion shows high expression in the center, in part of the inner ear (lower
right, below the scale bar), and in the ectoderm (left). The scale bar
represents either 100 .mu.m (C, F, and G) or 250 .mu.m (A, B, D, E, and H).

Detailed Description Text - DETX (82):

In situ hybridization analyses of LIMK1 expression in the embryonic human
nervous system demonstrated that LIMK1 is expressed in several discrete regions
of the brain and spinal cord (FIG. 6). In situ hybridization was performed on
6 mm-thick, paraffin embedded sections of freshly prepared human embryos, which
were obtained from the MRC-funded Human Embryonic Tissue Bank, Institute of
Child Health, London. A digoxigenin-labeled 625-bp cRNA probe specific to the
3'-untranslated portion of LIMK1 cDNA was used to avoid areas of homology with
other genes encoding proteins containing LIM and kinase domains; similar
results were obtained, however, in some sections hybridized with a cDNA probe
covering the kinase region and some of the 3'-untranslated sequence. The in

situ protocol was based on the detection of digoxigenin-labeled RNA by alkaline phosphatase-conjugated anti-DIG FAB fragments (Boehringer Mannheim), as previously described (Wilkinson, 1992; Birren et al., 1993). Brightfield microphotography was carried out with an Olympus BH-2 and Fujichrome 64T film.